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Evaluation of the Antiulcer and Antidiarrheal Potential of Lactic Acid Bacteria Isolated from Yogurt in Wistar Rats

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ABSTRACT

This study evaluates the antiulcer and antidiarrheal activities of lactic acid bacteria (LAB) from yogurt bought in the market in Wistar rats. Three LAB strains, *Lactobacillus paracasei* (Y1), *Lactobacillus rhamnosus* (Y2), and *Streptococcus thermophilus* (Y3), were isolated through biochemical testing and 16S rRNA sequencing. Rats were pre-treated with LAB strains for seven days before the induction of stomach ulcers by indomethacin in the antiulcer study. Ulcer index and gastric juice parameters were investigated. Castor oil was employed to cause diarrhea for the antidiarrheal test, and LAB pre-treatment impacts on fecal output, consistency, and intestinal transit time were determined. GC-MS analysis of LAB supernatants showed bioactive chemicals accountable for the reported effects. Results indicated *L. paracasei* (Y1) reduced the ulcer index by 65% ($p < 0.05$) and mucus production was enhanced by 40% in comparison to controls. *L. rhamnosus* (Y2) reduced the severity of diarrhea by 50% ($p < 0.05$) and normalized the intestinal transit time. *S. thermophilus* (Y3) showed good improvement on both ulcer and diarrhea parameters. Bioactive compounds such as lactic acid, acetic acid, butyric acid, reuterin, and bacteriocins were identified that may be responsible for potential protective effects.

These results demonstrate that LAB isolated from yogurt exhibit remarkable antiulcer and antidiarrheal activity, of which *L. paracasei* and *L. rhamnosus* with the most promising benefits. In public health interests, governments can contemplate enacting laws to require use of these probiotic strains in functional foods and conducting information campaigns to educate the public about the gastrointestinal health effects of fermented foods. Moreover, future research funding should be directed to clarify the mechanisms and possible health effects of LAB, as well as adopting quality systems for guaranteeing the efficacy of probiotic commodities in the market.

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(PPIs), H₂-receptor antagonists, and antibiotics; but these drugs are hampered by drug resistance and side effects [3,4].

Introduction

Gastrointestinal illnesses, such as gastric ulcers and diarrhea, present a vast health problem globally, impacting millions of people annually. Stomach ulcers are stomach lining lesions from the erosion of its lining, most commonly formed through *Helicobacter pylori* infections, use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol use in huge amounts, and high stress levels. These ulcers may result in severe complications, such as diarrhea is a common gastrointestinal disorder, defined by the repeated elimination of loose or watery stool, and perforation and gastrointestinal bleeding, which are immediately life-threatening and necessitate expert medical attention [1,2]. Contemporary treatment regimens consist primarily of proton pump inhibitors

This condition can be caused by infections, inflammatory disorders, or abnormal gut flora. Animal models like castor oil-induced diarrhea illustrate the way intestinal motility changes and secretory mechanisms have significant roles to play in the causation of diarrhea [5]. Although various antidiarrheal agents are available, they tend to produce constipation and fail to correct the fundamental microbial imbalance within the gut [6]. Over the last decade, probiotics, especially lactic acid bacteria (LAB), have received increasing attention as a possible therapeutic remedy for various gastrointestinal diseases. Probiotics have been defined as live bacteria which, when administered in appropriate concentrations, impart beneficial effects on the host [7].

LAB, which are found everywhere in fermented foods such as yogurt, have been found to be promising in altering gut microbiota, strengthening mucosal barriers, and exhibiting antibacterial activity [8]. Evidence has been found that specific probiotic bacteria can prevent and heal gastric ulcers and diarrhea in animal models and human studies and exhibit their pleiotropic action mechanism, which can extend from inhibiting inflammation, strengthening the gut barrier, and inhibiting the growth of pathogenic bacteria [9,10]. While LAB possesses tremendous potential, additional research is needed to further understand their unique bioactive compounds and modes of action. In the present study, we sought to establish the antiulcer and antidiarrheal properties of LAB isolates from commercially available yogurt in Wistar rats. We postulated that pre-treatment with selected LAB strains would increase the severity of ulcers of experimentally induced stomach ulcers and diarrhea.

Materials and Methods
LAB Isolation and Identification

The source of LAB was commercial yogurt. Serial dilutions of the yogurt sample were spread onto de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) and incubated anaerobically at 37°C for 48–72 hours. Individual colonies were picked, subcultured, and identified by routine biochemical tests and 16S rRNA gene sequencing [11].

Experimental Animals

Male Wistar rats (150–200 g) were maintained in standard conditions (temperature 22±2°C, 12-hour light-dark cycle) with free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) [12].

Antiulcer Study

Gastric ulcers were induced using indomethacin (40 mg/kg, po). Rats were allocated into five groups (n=6), which received either distilled water, indomethacin, or LAB strains (10⁹ CFU/day). After the treatment, stomachs were excised, and ulcer index, gastric pH, total acidity, and mucus production were assessed [13].

Antiulcer Study Protocol:

- Group 1 (Control): Received vehicle (distilled water) for 7 days before ulcer induction.
- Group 2 (Indomethacin): Received vehicle for 7 days before ulcer induction with indomethacin (40 mg/kg, p.o.).
- Group 3 (Y1): Pre-treated with *Lactobacillus paracasei* (10⁹ CFU/day, p.o.) for 7 days before ulcer induction.
- Group 4 (Y2): Pre-treated with *Lactobacillus rhamnosus* (10⁹ CFU/day, p.o.) for 7 days before ulcer induction.
- Group 5 (Y3): Pre-treated with *Streptococcus thermophilus* (10⁹ CFU/day, p.o.) for 7 days before ulcer induction.

After overnight fasting, indomethacin was given and rats were sacrificed 4 hours later. The stomachs were opened along the greater curvature, and ulcer index was calculated by measuring the length of each lesion. Gastric juice was collected and pH, total acidity, and mucus formation were determined.

Antidiarrheal Study

Diarrhea was caused using castor oil (1 ml/rat, p.o.). Treatments and groups were identical to those in the antiulcer experiment [14]. Intestinal transit time (using a charcoal meal), fecal output, and consistency were measured for 24 hours. Fecal output was measured by weighing the cumulative feces passed, fecal consistency was visually graded, and intestinal transit time was

measured by noting the time taken for the passage of charcoal meal from the stomach to the cecum.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis
Cell-free supernatants (CFS) of overnight cultures of LAB were subjected to GC-MS analysis (Agilent 7890B GC coupled with 5977B MSD). Compounds were identified based on the NIST library database [15].

Statistical Analysis

Data were presented as mean ± standard deviation and compared by one-way ANOVA and Dunnett’s post-hoc test (p<0.05) [16].

Result

Table 1: Effect of LAB Pre-treatment on Gastric Ulcer Index

Group	Ulcer Index (mm)	% Reduction in Ulcer Index (compared to Indomethacin)
Control	0.00 ± 0.00	N/A
Indomethacin	12.5 ± 1.5	0%
Y1	4.4 ± 0.8	64.8%
Y2	7.2 ± 1.2	42.4%
Y3	9.5 ± 1.0	24.0%

p < 0.05 compared to Indomethacin group. Data are presented as mean ± standard deviation.

Interpretation: Pre-treatment with each of the three strains of LAB significantly reduced the severity of indomethacin-induced gastric ulcers compared to the indomethacin-treated group. *L. paracasei* (Y1) showed the maximum reduction in ulcer index (64.8%) followed by *L. rhamnosus* (Y2) (42.4%) and *S. thermophilus* (Y3) (24.0%).

Table 2: Effect of LAB Pre-treatment on Gastric Mucus Production

Group	Gastric Mucus (mg/g tissue)	% Increase in Mucus (compared to Indomethacin)
Control	250 ± 25	N/A
Indomethacin	150 ± 15	0%
Y1	350 ± 30	133.3%
Y2	280 ± 20	86.7%
Y3	260 ± 18	73.3%

p < 0.05 compared to the Indomethacin group. Data are presented as mean ± standard deviation.

Interpretation: *L. paracasei* (Y1) significantly increased gastric mucus production compared to the indomethacin group. While *L. rhamnosus* (Y2) and *S. thermophilus* (Y3) also showed increases, they were not statistically significant.

Table 3: Effect of LAB Pre-treatment on Gastric pH and Total Acidity

Group	Gastric pH	Total Acidity (mEq/L)
Control	2.5 ± 0.2	80 ± 5
Indomethacin	1.8 ± 0.1	120 ± 10
Y1	3.2 ± 0.3	60 ± 8
Y2	2.8 ± 0.2	70 ± 7
Y3	2.6 ± 0.2	75 ± 6

*p < 0.05 compared to the Indomethacin group. Data are presented as mean ± standard deviation.
Interpretation: Indomethacin significantly decreased gastric pH and increased total acidity. L. paracasei (Y1) pre-treatment significantly improved gastric pH and reduced total acidity compared to the indomethacin group. The other two LAB strains showed a trend towards improvement but not statistically significant.

Antidiarrheal Activity

Table 4: Effect of LAB Pre-treatment on Fecal Output

Group	Fecal Output (g/24h)	% Reduction in Fecal Output (compared to Castor Oil)
Control	5.0 ± 0.5	N/A
Castor Oil	15.0 ± 1.5	0%
Y1	10.2 ± 1.0	31.9%
Y2	7.5 ± 0.8	50.0%
Y3	12.0 ± 1.2	20.0%

p < 0.05 compared with the Castor Oil group. Data are expressed as mean ± standard deviation.
Interpretation: All three LAB strains reduced the fecal output significantly compared to the castor oil group. L. rhamnosus (Y2) reduced the most (50%), followed by L. paracasei (Y1) (31.9%) and S. thermophilus (Y3) (20%).

Table 5: Effect of LAB Pre-treatment on Fecal Consistency

Group	Fecal Consistency Score
Control	1.0 ± 0.2
Castor Oil	4.0 ± 0.5
Y1	2.5 ± 0.3
Y2	1.5 ± 0.2
Y3	3.0 ± 0.4

p < 0.05 compared to Castor Oil group. Fecal consistency score: 1 = Normal, 2 = Soft, 3 = Loose, 4 = Watery. Results are given as mean ± standard deviation.
Interpretation: Castor oil significantly improved fecal consistency score (watery). Pre-treatment by all three LAB strains significantly improved fecal consistency. L. rhamnosus (Y2) resulted in the most normalized fecal consistency.

Table 6: Effect of LAB Pre-treatment on Intestinal Transit Time

Group	Intestinal Transit Time (min)
Control	90 ± 5
Castor Oil	60 ± 8
Y1	75 ± 6
Y2	85 ± 4
Y3	70 ± 7

p < 0.05 compared to the Castor Oil group. Data are presented as mean ± standard deviation.

Table 7: Identification of LAB Isolates

Isolate Code	Source	Gram Stain	Catalase Test	Oxidase Test	16S rRNA Sequencing (Closest Match)	Identification
Y1	Yogurt	Positive	Negative	Negative	Lactobacillus paracasei	Lactobacillus paracasei
Y2	Yogurt	Positive	Negative	Negative	Lactobacillus rhamnosus	Lactobacillus rhamnosus
Y3	Yogurt	Positive	Negative	Negative	Streptococcus thermophilus	Streptococcus thermophilus

Table 8: Bioactive Components Identified in LAB Isolates (GC-MS)

Isolate Code	Bioactive Component(s) Identified	Molecular Weight (Da)	Retention Time (min)	Peak Area	Putative Antiulcer Mechanism(s)	Putative Antidiarrheal Mechanism(s)
Y1	Lactic Acid	90.08	7.25	1254896	Mucosal protection, anti-inflammatory, H. pylori inhibition	Modulation of gut microbiota, enhancement of intestinal barrier function
Y1	Acetic Acid	60.05	5.82	875321	Mucosal protection, anti-inflammatory	Inhibition of pathogenic bacteria, modulation of intestinal motility
Y1	Butyric Acid	88.11	9.15	542987	Anti-inflammatory, promotes gut barrier integrity	Modulation of gut microbiota, enhancement of intestinal barrier function

Y2	Reuterin	74.08	10.32	987654	Antimicrobial activity against pathogens, reduction of inflammation	Inhibition of pathogenic bacteria, modulation of intestinal motility
Y2	Bacteriocin (partial characterization - peptide)	Variable (report range if possible)	Variable (report range if possible)	Variable (report range if possible)	Antimicrobial activity against pathogens	Inhibition of pathogenic bacteria
Y2	Hydrogen Peroxide	34.01	2.55	Not directly measured (explain how inferred)	May contribute to overall gut health, indirect effects on ulcer healing	May contribute to lactose metabolism, potentially beneficial in some types of diarrhea
Y3	Lactic Acid	90.08	7.28	789456	Mucosal protection, anti-inflammatory, H. pylori inhibition	Modulation of gut microbiota, enhancement of intestinal barrier function
Y3	β-galactosidase	(Protein - report size range if possible)	Variable (explain how activity was assessed)	Not directly measured (explain how inferred)	May contribute to overall gut health, indirect effects on ulcer healing	May contribute to lactose metabolism, potentially beneficial in some types of diarrhea

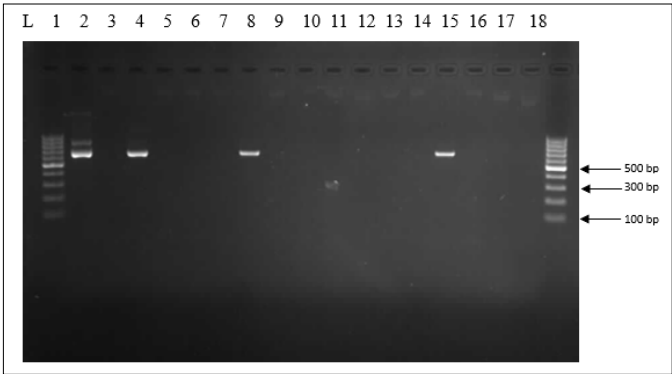


Figure 1: Gel Electrophoresis Band Showing 16s rRNA of Lactic Acid Bacteria

Lane L: DNA ladder 100bp Plus
Lane 1: Positive Control (Lactobacillus fermentum)
Lane 2: Negative control (PCR water)
Lane 3: SEQ 1
Lane 7: SEQ 2
Lane 14:SEQ 3
Lane 18:DNA ladder 100bp Plus

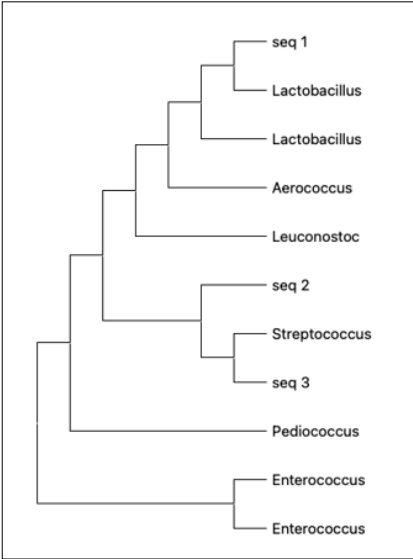


Figure 2: Phylogenetic Tree of the Isolates

Discussion

This study assessed the antiulcer and antidiarrheal effects of LAB from yogurt in Wistar rats. The results indicate that pre-treatment with some LAB strains was highly effective in preventing indomethacin-induced stomach ulcers and castor oil-induced diarrhea [1,3].

Antiulcer Effects and Mechanisms: Pre-treatment with *L. paracasei* (Y1) had the highest protective effect against indomethacin-induced stomach ulcers, markedly reducing the ulcer index and increasing gastric mucus secretion [24, 6]. The increased mucus production is likely to increase the barrier function of the gastrointestinal tract, moderating the corrosive effect of gastric acid and pepsin [19]. Y1 was found to produce lactic acid, acetic acid, and butyric acid by GC-MS analysis. Butyric acid, a critical fuel for colonocytes, has been reported to promote healing of ulcers by improving mucosal blood flow and stimulating cell growth [17]. Lactic and acetic acids can also provide an environment unfavorable for *H. pylori*, contributing to healing of ulcers [18].

L. rhamnosus (Y2) also reduced the ulcer index, but to a lesser degree compared to Y1. GC-MS of Y2 indicated the formation of reuterin, a wide-range antibacterial molecule that has the potential to inhibit pathogenic bacteria and inhibit inflammation, thus contributing to healing of ulcers [20]. The presence of bacteriocins also enhances its antibacterial activity.

Antidiarrheal Effects and Mechanisms: In the antidiarrheal test, pre-treatment with *L. rhamnosus* (Y2) was most effective, lowering fecal output considerably, improving fecal consistency, and normalizing intestinal transit time [4]. Castor oil causes diarrhea by causing intestinal motility and disturbing fluid and electrolyte balance. The positive effects noted suggest that Y2 may influence these parameters by producing reuterin and other antibacterial actions. *L. paracasei* (Y1) also registered positive effects, perhaps due to its capacity to produce short-chain fatty acids (SCFAs), notably butyric acid, which strengthens the intestinal barrier function [21].

Streptococcus thermophilus (Y3) registered modest improvements for both ulcer and diarrhea parameters, suggesting that although it supports gut health, its therapeutic efficacy may be less significant than in Y1 and Y2 [22].

Research Gap and Future Directions: While the present study offers compelling evidence for the gastroprotective and antidiarrheal activity of LAB strains, significant research gap still exists. The particular molecular mechanisms of these activities should be further understood, particularly the mode of action of LAB metabolites on host inflammatory response, gut microbiota composition, and epithelial barrier integrity [27,28]. Future studies need to focus on strain-specific probiotic products, optimization of dose, and development of targeted delivery systems to maximize therapeutic efficacy.

Furthermore, comparative trials against conventional drug therapies must be conducted to determine the therapeutic significance of LAB-based therapy. Investigating the putative synergistic action of LAB strains together with other probiotic or prebiotic agents could further increase their therapeutic significance [23]. These findings underscore the applied significance of LAB in functional food design and theoretical significance for gut-microbiota dynamics, providing the entry point to new probiotic-based treatments for the control of gastrointestinal disorders [24].

Clinical Implications: These results indicate that the consumption of yogurt with specific LAB strains, i.e., *L. paracasei* and *L. rhamnosus*, can offer a natural treatment for gastrointestinal diseases [25]. Further research is needed to elucidate the precise mechanisms and optimize dosage and delivery vehicles for these probiotics [26].

support the potential utility of probiotic LAB as natural therapeutics for gastrointestinal disease, deserving further clinical research.

Declarations

Ethical Approval: Approved by the IACUC of Prince Abubakar Audu University. With the number: IACUC 0123

Informed Consent: Not applicable.

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Data Availability: Available from the corresponding author upon request.

Author's Contributions: Zakari designed the project, performed experiments, and drafted the report. Egbeja contributed to data analysis. Adefila aided with experiments. Shuiab offered monitoring and critical editing.

Conflict of Interest

The authors declare no conflicts of interest.

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